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13. SUPPLEMENTARY NOTES

14. ABSTRACT
 During the reported period, we have been able to optimize the conditions for CCI. The goal of the optimization was to avoid overshooting, to diminish the depth of the impact and thus to reproducibly obtain and process brain tissue with minimal necrotic changes as close as possible to the impact. The experimental groups for both SAs are being generated. However, unexpectedly Abca1 knockout mice on human APOE genetic background were exceptionally difficult to generate. We are considering changes in the genotype of those particular groups, which has been mentions in the alternatives of the original proposal. The pilot mRNA-seq experiments are completed. The results demonstrated that metabolic and regulatory pathways relevant to inflammatory and immune reactions (of particular importance genes like Trem2 and Tyrobp), as well as Amyloid-beta clearance – critical for immediate and long term response to TBI can be modulated efficiently.

15. SUBJECT TERMS- Abca1 global deletion, APOE targeted replacement, complex breeding, CCI model optimization, mRNA library generation, high throughput massive parallel sequencing, analysis

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Contents

1 Introduction

Patients carrying apolipoprotein E4 (APOE) ?4 allele are more susceptible to poor neurological outcome after traumatic brain injury (TBI). Furthermore, the inheritance of APOe4 is the only proven genetic risk factor for sporadic Alzheimer disease (AD). Importantly, TBI is a risk factor for the subsequent development of AD particularly among APOE?4 carriers. ATP binding cassette transporter A1 (ABCA1) is a lipid transporter that controls the generation of HDL in plasma and ApoE-containing lipoproteins in the brain which are important for repairing axonal damage after TBI. We demonstrated that lack of Abca1 increases amyloid plaques and decreased APOE protein level in AD-model mice. In this proposal we will test the hypothesis that ABCA1 differentially affects the response of mice expressing human APOE isoforms to TBI. We will evaluate the immediate as well as the long-term effects following brain injury. The Aims are: 1) To determine the effect of Abca1 deficiency on the response to TBI in mice expressing human APOE3 and E4 isoforms, and 2) 2. To evaluate the development of AD-like phenotype in APP expressing mice exposed to TBI and to determine if the differences in the phenotype are mediated through ABCA1.

2 Keywords

Traumatic brain injury, APOE isoforms, ABCA1, Alzheimer disease, APP mice, amyloid beta, axonal injury, inflammatory reaction, trnascriptome, high-throughput massive parallel sequencing, mRNA-seq, behavioral testing, memory impairement, recovery.

3 Overall Project Summary

This section of the report shall be in direct alignment with respect to each task outlined in the approved SOW in a summary of Current Objectives, and a summary of Results, Progress and Accomplishments with Discussion.

During the Year One of the proposed project, the number of tasks as listed in the SOW was rather limited to the generation of groups of genetically modified mice optimization of the CCI and surgeries, initial behavioral tests and pilot mRNA-seq experiments. We emphasize, that the complicated genotypes require complex breeding strategies extended over a period of months, and those do not allow parallel testing of all experimental groups or even testing all mice of a single group during the same time period. Moreover, global deletion of ABCA1 has a pronounced effect on the reproduction, which inevitably translates in prolonged times to generate the experimental groups. Therefore, during the first year it was not possible to conduct experiments with genetically modified mice allowing statistical analysis and conclusions, which is clearly stated in the SOW. With the noticeable exception of APOE3/Abca1-ko and APOE4/Abca1-ko mice, all other groups are being generated as proposed.

3.1 Optimization of CCI model

One of the Objectives during the reported period was the optimization of the CCI model. There are two reasons that require this step: brain tissue processing for the proposed biochemical studies and mRNA-seq. The goal is to avoid overshooting, to diminish the depth of the impact and thus to be able to obtain and process no-necrotic brain tissue as close as possible to the impact.

The optimized conditions for CCI were applied to available, a limited number of APP/PS1 and APP/PS1/APOE4 mice and motor coordination deficits were evaluated in a beam walk test, rotarod and elevated plus maze 1, 2 or 3 days post-injury. While the number of the mice is too small and the data can not be used for reliable analyses and conclusions, based on the results, we will apply those optimized conditions for the future experiments, once the mice in the corresponding groups reach the age as proposed.

3.2 RNA processing and mRNA-seq

One of the most important tasks throughout the proposed study is to reveal changes in the brain transcriptome of injured genetically modified mice that would allow conclusions about the role of LXR/RXR-ABCA1-APOE regulatory axis in the response to and the recovery from TBI. At the end of the reported period, we completed the pilot, initial set of experiments with brain tissue from uninjured APP/PS1 mice. We used cortices from two groups of 4 animals each - Bexarotene and vehicle treated and performed high throughput massively parallel sequencing on mRNA libraries. Bexarotene is a small molecule RXR ligand, FDA approved for treatment of certain types of skin cancer.

Trough activation of LXR/RXR transcription factors this ligand causes up regulation of Abca1 and Apoe. This effect rationalized the application of the drug in the pilot experiments. The pilot experiments unexpectedly generated extremely important data revealing regulatory and metabolic networks that are considered crucial in response to TBI and neurodegeneration, and the same time demonstrating the possibility for pharmacological modulation with therapeutic effect. These findings can be summarized in the following way:

- Gene Ontology (GO) Biological Process categories with the highest fold enrichment and lowest False Discovery Rate (FDR) clustered in immune response, inflammatory response, defense response and immunoglobulin mediated immune response GO terms.

- We found up-regulation of the genes Trem2, Tyrobp, C1q complex and Ttr, considered highly important for risk, development and progression of neurodegeneration in the context of inflammatory reactions and A-beta clearance
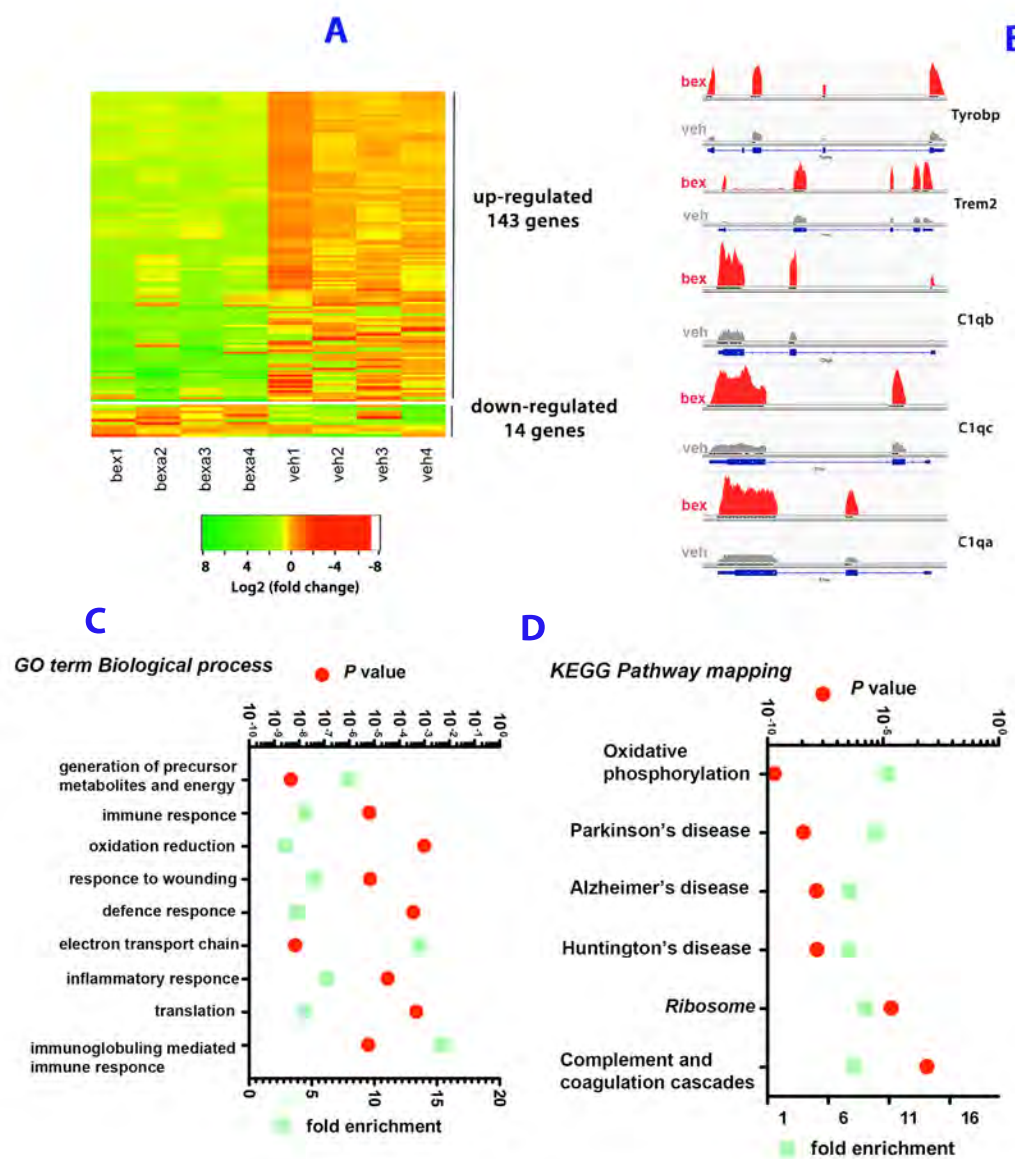

Figure 1

3.3   Actual and Anticipated problems

By the end of the reported period of the award we can clearly outline 2 major problems.

A) Generation of mice with global deletion of *Abca1* on human APOE background. The generation of experimental groups of some genotypes has been and remains a difficult step. At this point we refer to specific genotypes of mice, not to experimental treatment groups. While the generation of mice expressing human APP and globally deleted *Abca1* is a well know problem since 2004, the breeding of ApoE4 and E3 targeted replacement mice to *Abca1^{ko}* turned out particularly difficult. The reason has been the very low number of, or no offspring of ApoE4/*Abca1^{ko}*, or ApoE3/*Abca1^{ko}* regardless of the size of the litters. At this time we don't have an answer what is the reason for the problem. Based on our previous experience, insisting on generation of those genotypes is cost-ineffective. Moreover, APOE4/*Abca1^{ko}* or APOE3/*Abca1^{ko}* is not a human relevant model, although we recognize that complete lack of ABCA1, compared to Abca1 heterozygosity, may be a better choice to demonstrate the role of functional ABCA1 in immediate and long term effects following TBI. Therefore we are seriously considering replacing APOE3(4)/*Abca1^{ko}* mice with APOE3(4)/ *Abca1^{het}*. We will deliver the final decision in the next quarterly report.

B) Inability to have un-degraded RNA following CCI is the second serious problem, that requires swift decision and proper changes. The problem with CCI in principle has been anticipated in the original proposal and an alternative was considered. At this moment we are trying to answer the question how far from the impact and within the penumbra we can obtain high quality RNA, or if not how far we have to go beyond the penumbra. The experimental set up is RT-QPCR and evaluation of expression level of genes based on our results with mRNA-seq. The conclusions from these ongoing experiments by themselves will be very important.

4   Key Research Accomplishments

- High throughput massive parallel sequencing - mRNA-seq, for evaluation of changes in transcriptome can be performed successfully using brain tissue from WT and genetically modified mice.

- Crucial in the response to TBI metabolic pathways revealed by mRNA-seq and known to be be regulated by activated LXR/RXR transcription factors can be modulated pharmacologically with therapeutic impact.

5   Conclusion

Successful accomplishment of the initial mRNA-seq experiments provide the background for conducting the next series of tasks with mice exposed to TBI. The results of the mRNA-seq assays, even not extensive, allow the exploration of pharmacological agents for therapeutic interventions in in the immediate, post TBI terms.

6   Publications, Abstracts, and Presentations

1. Peer-Reviewed Scientific Journals:
   Koldamova R, Fitz NF, Lefterov I., ATP-binding cassette transporter A1: From metabolism to neurodegeneration. **Neurobiol Dis. 2014 Dec;72PA:13-21.** (http://dx.doi.org/10.1016/j.nbd.2014.05.007 Epub 2014 May 17.

7   Inventions, Patents and Licenses

NA

8   Reportable Outcomes

NA

9   Other Achievements

NA

10   References

NA

11   Appendices

NA